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Differential expression of ABC transporters and their regulatory genes during lactation and dry period in bovine mammary tissue

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ATP-binding cassette (ABC) transporters play a pivotal role in human physiology, and mutations in these genes often result in severe hereditary diseases. ABC transporters are expressed in the bovine mammary gland but their physiological role in this organ remains elusive. Based on findings in the context of human disorders we speculated that candidate ABC transporters are implicated in lipid and cholesterol transport in the mammary gland. Therefore we investigated the expression pattern of selected genes that are associated with sterol transport in lactating and nonlactating mammary glands of dairy cows. mRNA levels from mammary gland biopsies taken during lactation and in the first and second week of the dry period were analysed using quantitative PCR. Five ABC transporter genes, namely *ABCA1*, *ABCA7*, *ABCG1*, *ABCG2* and *ABCG5*, their regulating genes *LXRα*, *PPARγ*, *SREBP1* and the milk proteins *lactoferrin* and *α-lactalbumin* were assessed. A significantly enhanced expression in the dry period was observed for *ABCA1* while a significant decrease of expression in this period was detected for *ABCA7*, *ABCG2*, *SREBP1* and *α-lactalbumin*. *ABCG1*, *ABCG5*, *LXRα*, *PPARγ* and *lactoferrin* expression was not altered between lactation and dry period. These results indicate that candidate ABC transporters involved in lipid and cholesterol transport show differential mRNA expression between lactation and the dry period. This may be due to physiological changes in the mammary gland such as immigration of macrophages or the accumulation of fat due to the loss of liquid in the involuting mammary gland. The current mRNA expression analysis of transporters in the mammary gland is the prerequisite for elucidating novel molecular mechanisms underlying cholesterol and lipid transfer into milk.

Keywords: ABC transporter, dry period, gene expression, lactation, mammary gland, nuclear receptors, SREBP.

The ATP-binding cassette transporter (ABC transporter) superfamily is one of the largest and most ancient families with representatives in all existing phyla from prokaryotes to man. ABC transporters utilize the energy of ATP hydrolysis to transport a wide variety of substrates across cellular membranes, including metabolites, lipids, sterols and drugs (Higgins, 1992; Dean & Allikmets, 1995). The classification as ABC transporter is based on the sequence and organization of the ATP-binding domains, also known as nucleotide-binding folds (NBFs). ABC transporters play a central role in the development of multidrug resistance (MDR). This is caused by several factors, one of which is increased excretion of the drug from the cell by ABC transporters. For example, ABCB1 and ABCG2, also known as P-glycoprotein and breast cancer resistance protein (BCRP), respectively, give resistance to therapeutically relevant drugs such as topotecan, irinotecan and doxorubicin (Allen & Schinkel, 2002; Di Nicolantonio et al. 2005).

More recently ABC transporters were identified that are implicated in the translocation of lipids. They were found either on the basis of homology with known transporters or as causative genes in disease loci. Hence, the function of ABCA1 and ABCG1 is to export excess cellular cholesterol into the HDL pathway and reduce cholesterol accumulation in macrophages (Oram & Vaughan, 2006). ABCA7 was demonstrated to mediate a similar reaction as ABCA1

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to generate HDL in vitro and it may be involved in lipid metabolism in kidneys and adipose tissues (Wang et al. 2003; Linsel-Nitschke et al. 2005; Kim et al. 2005). ABCG5 and ABCG8 are highly expressed in the intestine and liver cells where they form heterodimers that limit the absorption of dietary sterols in the intestine and promote cholesterol elimination from the body through hepatobiliary secretion (Yu et al. 2002; Mutch et al. 2004).

The expression of several ABC transporters is under tight transcriptional regulation and orphan nuclear receptors play an important role in this context. Nuclear receptors comprise a family of transcription factors that act as heterodimers, which bind to promoter elements and induce gene expression. The retinoid X receptor (RXR) is a compulsory partner in the heterodimer. The other partner ascertains the specificity for the activating ligand and for the target gene (Di et al. 1999). Other genes relevant for regulating the expression of ABC transporters involved in lipid and carbohydrate metabolism are the liver X receptors (LXR) α and β, the peroxisome proliferator-activated receptors (PPAR) α and γ and the sterol regulatory element binding proteins (SREBP) 1 and 2.

Whereas ABC transporters play a substantial role in hereditary human diseases, only scarce information is available about their expression and function in food-producing animals. Only few ABC proteins have been identified in *Bos taurus* (Ambagala et al. 2002; Taguchi et al. 2002; Beharry et al. 2004; Cohen-Zinder et al. 2005; Farke et al. 2006; Viturro et al. 2006) and their functions remain mostly speculative.

The aim of the current study was to detect ABC transporters that are known to play a role in lipid transport in the bovine mammary gland. The demonstration of transporter gene levels in the lactating mammary gland may identify candidate transporters involved in lipid homeostasis in the lactating mammary gland. Furthermore, expression levels of these transporters in lactating as compared with nonlactating mammary gland tissue may identify a subset of transporters involved in lipid and cholesterol transport into milk.

Materials and Methods

Cows and their management

This study was performed according to the requirements of the Bavarian state animal welfare committee (Germany).

Four healthy dairy cows (German Braunvieh), two of them in their first and two in their second lactation, free of clinical udder health problems were used. At the beginning of the experiment, two cows were in early lactation (4–100 d) and two were lactating for more than 300 d. The animals were kept in a loose-housing barn and milked twice daily at 5.00 and 17.00 in a milking parlour. Feeding consisted of maize and grass silage, hay and concentrate according to their individual levels. Water was available ad libitum.

Biopsy procedure

Biopsies were carried out after the morning milking. The experimental quarters were clipped and cleaned. Cows were mildly sedated with an i.v. injection of 0·8 ml xylazine (2%, CP-Pharma, Burgdorf, Germany). Biopsy samples from the rear quarters were taken from a caudal direction and the biopsy site was carefully selected to avoid the cisternal region and larger subcutaneous blood vessels. The area was washed, sterilized with 70% ethanol and then anaesthetized by a s.c. injection of 3·5 ml lidocaine (2%, Chassot, Ravensburg, Germany). Thereafter a puncture incision was made through the skin with a sterile single-use scalpel and one or two biopsies of one single quarter were taken through this incision using a human Bard®Magnum™ Biopsy Instrument (BARD, Covington, USA) and a Bard®Magnum™ Core Tissue Biopsy Needle (12 g × 10 cm) (BARD). A core of maximum 20 mg mammary tissue was extracted. Until the next morning a swab was fixed with an adhesive Fixomull® stretch plaster (Beiersdorf AG, Hamburg, Germany) to avoid contamination. Mammary biopsies were taken as described above for a total of nine consecutive days as well as one and two weeks after onset of the dry period. Neither during nor after the experiment did the cows need anti-inflammatory or antibiotic therapy. The wound dressing was removed 2 d after taking the last biopsy.

RNA isolation and cDNA synthesis

The tissues were homogenized with an Ultra-Turrax (IKA®-WERKE, Staufen, Germany). Total RNA of mammary gland biopsies was isolated using TriPure (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendations. To quantify the amount of total RNA extracted, optical density (OD) was determined. The integrity of the RNA was verified by the OD_{260}/OD_{280} absorption ratio >1·8.

Synthesis of first strand complementary DNA (cDNA) was performed with reverse transcriptase (SuperScript II, Invitrogen, Karlsruhe, Germany) and random hexamer primers according to the manufacturer's instructions.

Oligonucleotide primers and PCR

All primer pairs (Table 1) were designed according to bovine or human nucleic acid data bank sequences and covered one or two exon boundaries to avoid amplification of genomic DNA. To test specificity of the primers and to evaluate the optimal annealing temperature, all primer pairs were initially tested by gradient PCR using mammary gland cDNA. PCR reactions were performed in a PCR thermocycler (Biometra, Göttingen, Germany) and contained 50 ng of cDNA, 12·5 µl 2 × PCR Master Mix (Promega, Madison, USA), 0·8 µM of forward and reverse primer (Metabion, Martinsried, Germany) and nuclease-free water in a final volume of 25 µl. PCR products were

Table 1. Primer sequences and amplification product sizes of reference and target genes

Gene	Forward primer	Reverse primer	Product size
GAPDH	5′-GTCTTCACTACCATGGAGAAGG-3′	5′-TCATGGATGACCTTGGCCAG-3′	197 bp
Ubiquitin	5′-AGATCCAGCATAAGGAAGGCAT-3′	5′-GCTCCACCTCCAGGGTGAT-3′	198 bp
β-Actin	5′-AACTCCATCATGAAGTGTGACG-3′	5′-GATCCACATCTGCTGGAAGG-3′	214 bp
Lactoferrin	5′-GAACATCCCCATGGGCCTG-3′	5′-CAGCCAGGCACCTGAAAGC-3′	203 bp
α-Lactalbumin	5′-ACCAGTGGTTATGACACACAAGC-3′	5′-AGTGCTTTATGGGCCAACCAGT-3′	233 bp
LXRα	5′-CTGCGATTGAGGTGATGCTC-3′	5′-CGGTCTGCAGAGAAGATGC-3′	229 bp
PPARγ	5′-CTCCAAGAGTACCAAAGTGCAATC-3′	5′-CCGGAAGAAACCCTTGCATC-3′	198 bp
SREBP1	5′-GACGGCCAGGTGAATCCAGA-3′	5′-CAGGACCATCTCTGCCCTCA-3′	217 bp
ABCA1	5′-GGACATGTGCAACTACGTGG-3′	5′-TGATGGACCACCCATACAGC-3′	134 bp
ABCA7	5′-GCCCAGGTCAACCGAACT-3′	5′-AGCACGAAGAGCTTCCACTC-3′	201 bp
ABCG1	5′-GACTCGGTCCTCACGCAC-3′	5′-CGGAGAAACACGCTCATCTC-3′	203 bp
ABCG2	5′-GCTCCTGAAGAGGATGTC-3′	5′-CAGCGGAAACCTATGGCTC-3′	174 bp
ABCG5	5′-AGCTCAGGCTCAGGGAAAAC-3′	5′-GTCGCTCTGCAGGACGTAG-3′	188 bp

Table 2. PCR conditions for quantitative PCR measurements of reference and target genes

	Denaturation		Primer annealing		Elongation		Fluorescence acquisition	
	T, °C	time, s	T, °C	time, s	T, °C	time, s	T, °C	time, s
GAPDH	95	15	58	10	72	20	†	
Ubiquitin	95	15	60	10	72	20	†	
β-Actin	95	15	62	10	72	20	†	
Lactoferrin	95	15	61	10	72	20	87	3
α-Lactalbumin	95	15	59	10	72	20	†	
LXRα	95	15	61	10	72	20	†	
PPARγ	95	15	63	10	72	20	83	3
SREBP1	95	15	60	10	72	20	87	3
ABCA1	95	15	55	10	72	20	†	
ABCA7	95	15	59	10	72	20	†	
ABCG1	95	15	57	10	72	20	†	
ABCG2	95	15	54	10	72	20	†	
ABCG5	95	15	60	10	72	20	†	

† Fluorescence acquisition was performed at the end of each elongation cycle

subjected to gel electrophoresis in 2 % agarose gels containing 0·05 µl of GelRed™ (Biotium, Hayward, USA) per ml. The DNA fragments were extracted using the Wizard SV Gel and PCR Clean-Up System (Promega), and both strands were commercially sequenced (Agowa, Berlin, Germany).

Real-time PCR

Quantitative real-time PCR (qPCR) in bovine mammary gland tissue was carried out using LightCycler® DNA Master SYBR Green technology (Roche Diagnostics). PCR reactions were performed in a final volume of 10 µl, using 1 µl of the LC FastStart DNA Master SYBR Green I (Roche Diagnostics), 4 pmol of each primer, 3 mM-$MgCl_2$, and 50 ng of cDNA. Before amplification, an initial high-temperature incubation step was performed to activate the DNA polymerase and to ensure complete denaturation of cDNA. Product-specific PCR conditions are listed in Table 2. Amplified products underwent melting curve analysis after the last cycle to specify the integrity of amplification. Data were analysed using the Second Derivate Maximum calculation described in the LightCycler® Software 3.5. All runs included a negative cDNA control consisting of PCR-grade water, and each sample was measured in duplicate.

Data analysis and statistics

Quantitative real time PCR data were processed using the relative quantification ΔΔCT-method ($2^{-\Delta\Delta CT}$ (Livak & Schmittgen, 2001). Expression changes are shown as relative up- or down-regulation compared with lactation (Table 3). Data were obtained as CT values, which represent the cycle number at which logarithmic plots cross a calculated threshold. For normalization of target gene (TG) expression, the CT values of the reference genes (RGs) *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*),

Table 3. Gene expression differences in nonlactating relative to lactating mammary glands. Values indicate n-fold expression ($2^{-\Delta\Delta CT}$) ± SEM in lactation as compared with dry period 1 (D1, 1 week after drying off) and dry period 2 (D2, 2 weeks after drying off). A value of 1 indicates no change in relative expression, values >1 and <1 indicate elevated and decreased mRNA expression levels, respectively

	Expression change as compared with lactation phase, L			
	D1	*P* value	D2	*P* value
ABCA1	3·12 ± 0·61	*P*=0·06	2·84 ± 0·37	*P*=0·04
ABCA7	0·08 ± 1·76	*P*=0·08	0·04 ± 0·95	*P*=0·03
ABCG1	1·56 ± 0·69	*P*=0·56	0·42 ± 2·50	*P*=0·65
ABCG2	0·16 ± 0·59	*P*=0·22	0·07 ± 0·42	*P*=0·04
ABCG5	0·11 ± 0·82	*P*=0·18	0·13 ± 1·07	*P*=0·07
PPARγ	3·88 ± 2·70	*P*=0·32	0·93 ± 2·64	*P*=0·92
LXRα	0·66 ± 2·66	*P*=0·85	3·84 ± 1·45	*P*=0·27
SREBP1	0·13 ± 0·99	*P*=0·02	0·14 ± 0·66	*P*=0·04
Lactoferrin	2·12 ± 3·57	*P*=0·78	2·33 ± 2·23	*P*=0·67
α-Lactalbumin	0·03 ± 1·95	*P*=0·21	0·0004 ± 1·37	*P*=0·01

β-actin and *ubiquitin* were averaged and the mean value served as reference gene index. The following calculations were performed:

$$\Delta CT = CT_{(RG)} - CT_{(TG)}$$

$$\Delta\Delta CT = \Delta CT_{(\text{dry period})} - \Delta CT_{(\text{lactation})}$$

$$\Delta CT_{(\text{lactation})} = \text{mean } \Delta CT \text{ value of four cows during lactation}$$

$$\Delta CT_{(\text{dry period})} = \text{mean } \Delta CT \text{ value of four cows during dry period}$$

SigmaPlot software (Systat Software Inc., San Jose CA, USA) was used for statistical analysis.

To analyse differences in the expression levels of the RGs, CT values were compared using a *t* test. For the TGs the expression changes were analysed at the ΔCT levels with a paired *t* test to exclude potential bias because of averaging data that had been transformed through the equation $2^{-\Delta\Delta CT}$. A *P* value <0·05 was regarded as statistically significant. The linear regression was calculated using the regression wizard included in the SigmaPlot software.

Results

Mammary gland biopsies from nine consecutive days during lactation and after the first and second week after dry off, respectively, were analysed. To determine whether lactation altered transporter gene expression, individual transporter RNA expression levels were compared in lactating and nonlactating bovine mammary glands. For normalization the arithmetic mean of the CT values originating from the three housekeeping genes *GAPDH*, *β-actin* and *ubiquitin*, was used. The mean of the housekeeping genes showed no significant differences between lactation and dry period (*P*=0·1572, data not shown). However, as *β-actin* represents not an optimal housekeeper in the mammary gland, all expression profiles were also evaluated without *β-actin*, i.e., data were normalized to the mean of the two housekeepers GAPDH and *ubiquitin*. The final outcome, however, remained essentially unchanged (data not shown).

Table 3 summarizes the n-fold differences in target gene RNA expression levels between lactating and nonlactating mammary glands. There was a significant difference in expression between lactation and dry period for *ABCA1*, *ABCA7, ABCG2, SREBP1* and *α-lactalbumin* (Fig. 1, Table 3). Within the first week after dry off (D1) an increase of *ABCA1* gene expression was observed which reached statistical significance (*P*=0·0439) in the second week (D2). A significant decrease of *SREBP1* gene expression was detected in the first (D1, *P*=0·0204) and second (D2, *P*=0·0435) week of the dry period. *ABCA7* and *ABCG2* expression decreased at the beginning of dry period (D1) and declined significantly (*P*=0·0323 and *P*=0·0382, respectively) in the second week (D2). Likewise, the expression of *α-lactalbumin* showed a declining trend within the first (D1) and a significant decrease (*P*=0·0113) in the second week of dry period (D2).

For *LXRα* and *lactoferrin* an increase in gene expression within the dry period was observed while for *ABCG5* a decreased expression level, from the lactating to nonlactating state was demonstrated. These trends, however, were not statistically significant.

All genes tested, except *ABCG1* and *PPARγ* for which no apparent changes in gene expression during lactation and involution were observed, showed a clear trend towards significance in the second week of dry period (Table 3).

As LXRα, PPARγ and *SREBP1* are known to be implicated in the regulation of ABC transporters involved in lipid homeostasis, we performed regression analysis with the relative gene expression values of the ABC transporters

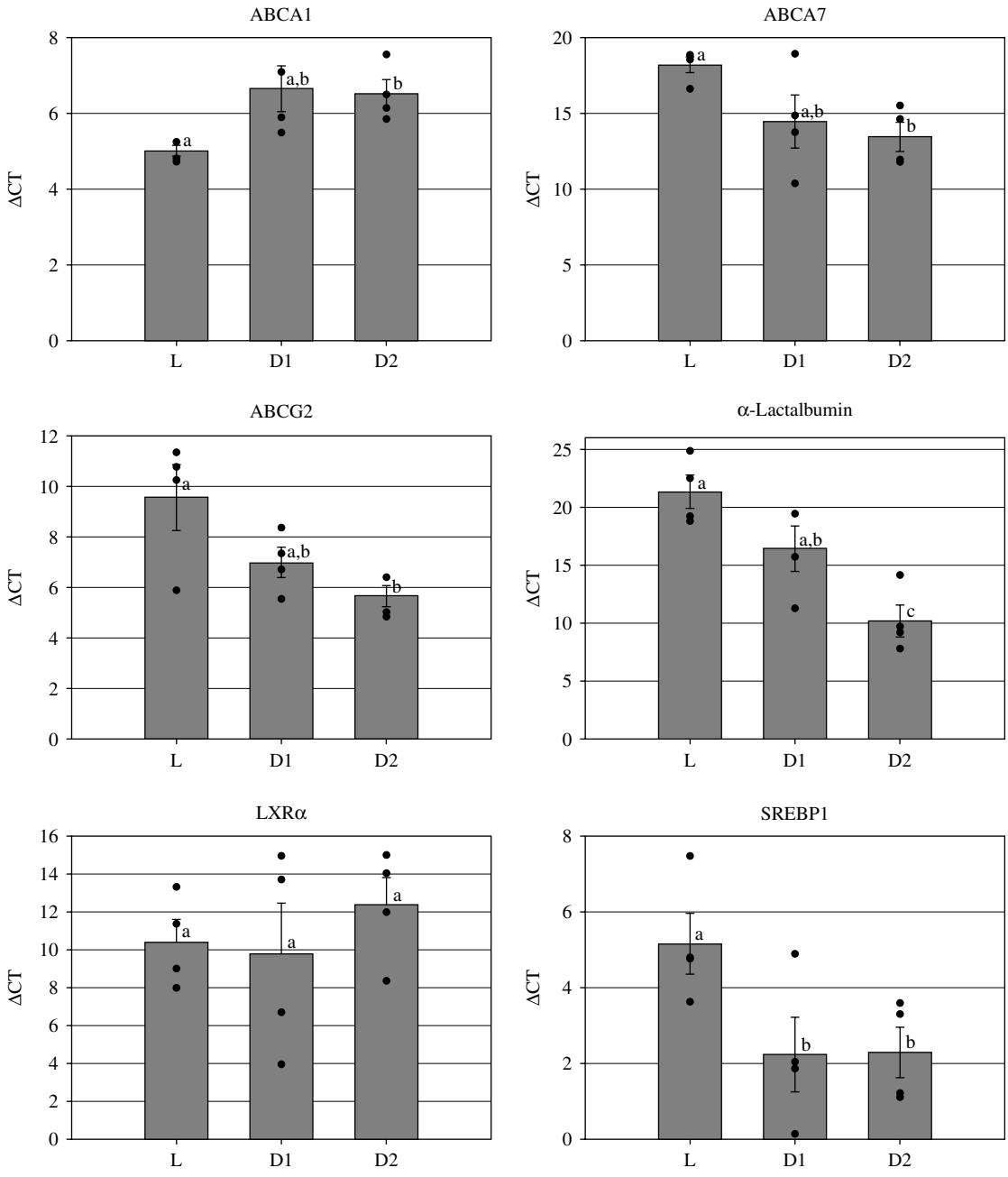

Fig. 1. Changes in mRNA expression levels of ABCA1, ABCA7, ABCG2, α-lactalbumin, LXRα and SREBP1 in bovine mammary glands between lactation and dry periods. L = normalized mean (ΔCT values) for lactation; D1 = normalized mean (ΔCT values) for the first week of the dry period; D2 = normalized mean (ΔCT values) for the second week of the dry period. Error bars indicate SEM. Means without a common letter are significantly different ($P<0{\cdot}05$).

and these factors. We observed a similar trend for *ABCA1* and *LXRα* (Fig. 2A), *ABCA7* and *SREBP1* (Fig. 2B) and *ABCG5* and *SREBP1* (Fig. 2C) gene expression, but for none of the other genes tested (data not shown). The relationship between *ABCA1* and *LXRα, ABCA7* and *SREBP1* and *ABCG5* and *SREBP1* showed a correlation coefficient (r) of 0·82 (Fig. 2A, insert), 0·91 (Fig. 2B, insert) and 0·95 (Fig. 2C, insert), respectively.

Discussion

The active involution process in cows begins with the cessation of regular milk removal, either by drying off or by weaning the calf, and is probably complete by 21–30 d after dry off. It is a transition phase of the mammary gland from the lactating to the nonlactating state; milk continues to accumulate for a couple of days after drying

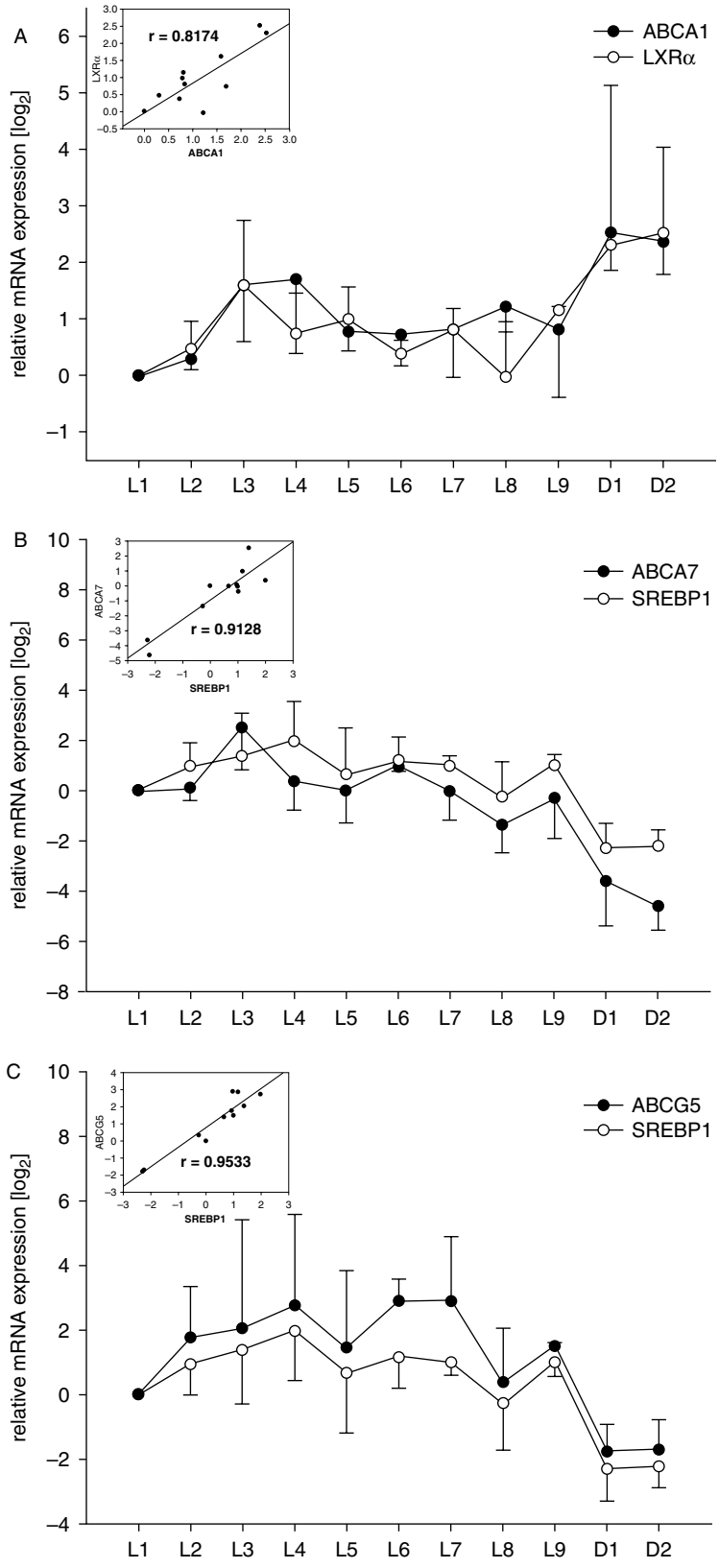

Fig. 2. Relative mRNA expression levels (ΔΔCT values) of (A) ABCA1 and LXRα, (B) ABCA7 and SREBP1 and (C) ABCG5 and SREBP1 during nine consecutive days in lactation (L1–L9) and after dry off (D1, D2) with regression lines and correlation coefficients (inserts). L = lactation; D1 = first week of the dry period; D2 = second week of the dry period; *r* = correlation coefficient. Error bars indicate SEM.

off. A significant reduction in fluid volume in the gland occurs between days 3 and 7 of involution (Hurley, 1989). However, it is also known that some cows involute very slowly after non-milking (C Farke, HHD Meyer, RM Bruckmaier and C Albrecht, unpublished observations). These factors may explain our observation of marked differences in gene expression levels predominantly in the second week of the dry period.

Concentrations of milk-specific components such as caseins, α-lactalbumin, β-lactoglobulin, and milk fat decline during the first 2–3 weeks of the dry period (Hurley & Rejman, 1986). In agreement with these findings we observed a significant decrease in *α-lactalbumin* gene expression, confirming adequate biopsy sampling procedures and mRNA measurements and demonstrating that our samples are representative for analysing expression differences between the lactation and dry period (Fig. 1). However, it cannot be completely excluded that, owing to the biopsy sampling technology, varying proportions of peripheral tissues may be included and that the limited numbers of biopsies collected may not completely reflect the majority of the tissue because of local variations. These factors may indicate that very subtle variations in gene expression may not be significantly identified.

The expression of *lactoferrin*, another control gene in this experiment, is regulated differently from that of other milk proteins. An increase of *lactoferrin* gene expression was observed during the first and second week of the dry period, which is in concordance with reports showing that *lactoferrin* is very low in bovine milk during mature lactation and is markedly elevated during mammary involution (Goodman & Schanbacher, 1991). This trend, however, did not reach statistical significance presumably owing to the low number of animals included in this study and a high interindividual variability between the cows. Indeed, when lactoferrin expression profiles were analysed in the context of every single cow, three of the four animals showed the expected increase in *lactoferrin* expression during the dry period while one animal exhibited an opposite trend (data not shown). This could indicate that also for other genes measured in this study a closer association between expression changes might be detected if the results across the genes examined were compared within each animal separately as opposed to using combined averaged data.

Having principally confirmed that our sampling procedures and RNA measurements were adequate and reliable, we focused on the expression pattern of selected ABC transporters involved in lipid, phospholipid and cholesterol transport. We hypothesized that the major structural, morphological and functional differences which occur during and after the onset of the involution period might lead to marked differences in the gene expression levels of candidate ABC transporters implicated in lipid homeostasis in the mammary gland. We therefore compared gene expression levels of ABCA1, ABCA7, ABCG1 and ABCG5 in lactating and nonlactating mammary glands.

It has been reported that milk cholesterol is partially synthesized in the mammary gland but that the major proportion is mainly derived from serum cholesterol (Long et al. 1980). The mechanism by which serum cholesterol is transferred into the milk is still unclear. In human physiology it is well established that ABCA1 represents a cholesterol efflux regulatory gene that plays a major role in the biosynthesis of high density lipoprotein and reverse cholesterol transport (Oram & Vaughan, 2006). ABCA1 is highly expressed in tissue macrophages (Lawn et al. 2001) and it has been reported that *ABCA1* transcripts are up-regulated in macrophages involved in the engulfment and clearance of apoptotic cells (Luciani & Chimini, 1996). We have demonstrated that in bovine tissues ABCA1 is predominantly expressed in lung, oesophagus, uterus, spleen and muscle (Farke et al. 2006). Our present study revealed that ABCA1 gene expression was significantly up-regulated in bovine mammary glands during the dry period. It is possible that this increase of *ABCA1* expression in the nonlactating mammary gland could be associated with the reported immigration of macrophages during involution (Monks et al. 2002). To test this hypothesis we measured mRNA levels of the macrophage-specific *CD14* gene in our mammary gland samples. We observed high inter-individual variation in CD14 expression especially in the dry period and found a trend towards an elevated expression in the first week of the dry period (D1; data not shown). This tendency, however, did not reach statistical significance and should be tested not only with more animals but should also be evaluated on protein level. Interestingly, macrophages from involuting sheep mammary glands apparently have phagocytic vacuoles containing casein micelles, lipid droplets and cellular debris (Tatarczuch et al. 2000). This suggests that these cells play a role in clearance of residual milk and fragmented death cells. Whether ABCA1 could be implicated in cholesterol and phospholipid transport or intracellular trafficking in the mammary gland is currently unclear. Fong et al. (2007) recently identified apolipoprotein (apo) E and apoAI, a key acceptor of cholesterol effluxed by ABCA1 (Oram et al. 2000) in bovine milk fat globule membranes. These findings indicate that potential molecular acceptors for ABCA1-meditated cholesterol efflux are present in bovine milk. However, to shed light on the physiological role of ABCA1 in the mammary gland, it is crucial to determine its cellular localization and to investigate whether ABCA1 is expressed in milk fat globules or other intracellular compartments.

Surprisingly, the expression patterns of *ABCA1* and *ABCA7* in the bovine mammary gland showed opposite trends (Fig. 1). While *ABCA1* was up-regulated, *ABCA7* expression decreased during the dry period. ABCA1 is induced by cholesterol through the LXR system (Venkateswaran et al. 2000) whereas ABCA7, which is highly homologous to ABCA1, is negatively regulated by cellular cholesterol (Iwamoto et al. 2006). Wang et al. (2003) demonstrated that, in contrast to *ABCA1*, *ABCA7*

shows moderate basal mRNA and protein levels in macrophages and no induction by LXR. These studies also show that ABCA7 has the ability to bind apolipoproteins and promote efflux of cellular phospholipids without cholesterol, suggesting a possible role of ABCA7 in cellular phospholipid metabolism in peripheral tissues. This indicates that the high homology between ABCA1 and ABCA7 may not be extrapolated to physiological functions. The physiological role of ABCA7 in the mammary gland currently remains elusive.

Similarly to ABCA7, ABCG5 showed a decreased expression in the dry period (Table 3) which, however, did not reach statistical significance. Our laboratories have previously demonstrated high mRNA expression of ABCG5 and ABCG8 in the bovine liver and digestive tract and, interestingly, in the lactating mammary gland (Viturro et al. 2006). However, in the present set of samples ABCG5 expression was significantly lower, with CT values mostly ranging between 30 and 35. Thus the data gained in these experiments should be interpreted with caution and do not currently allow us to postulate an important role for these genes in the mammary gland.

In parallel with the above mentioned lipid transporters, we also measured ABCG2 expression in our mammary gland samples. Jonker et al. (2005) demonstrated that the ABCG2 transporter is strongly induced in the mammary gland of mice, cows and women during lactation and that it is responsible for the active secretion of clinically and toxicologically important substrates into mouse milk. They observed that during involution, *ABCG2* expression declined rapidly. In agreement with these data and other studies (Cohen-Zinder 2005) our investigations revealed a significant decrease of *ABCG2* expression from the lactating to the non-lactating state in the bovine mammary gland (Fig. 1). It is currently unclear why and to what extent ABCG2 is functionally active in the mammary gland. Therefore it is essential to identify physiological ligands for ABCG2 and to investigate which of them may account for the high expression during lactation. In this context, van Herwaarden et al. (2007) recently demonstrated that ABCG2 not only secretes drugs but also riboflavin (vitamin B_2) into milk, implying that vitamin B_2 might represent an endogenous ligand for ABCG2 in the mammary gland. Interestingly, a missense mutation in the ABCG2 gene was recently found to affect milk yield, milk fat and protein concentration in cattle (Cohen-Zinder, 2005) suggesting a functional role for ABCG2 in milk secretion.

Expression of several ABC transporters, especially those implicated in lipid homeostasis, is regulated by transcription factors such as nuclear receptors and SREBPs. We analysed the expression of *PPARγ*, *LXRα* and *SREBP1* to investigate a potential correlation between the transporters and their regulators. We observed a similar expression pattern for *ABCA1* and *LXRα* (Figs 1 and 2A) suggesting that LXRα is involved in the regulation of ABCA1 expression in the bovine mammary gland. Indeed LXRα was 3·84±1·45 fold increased in the second week of the dry period (Table 3). However, probably owing to the low number of animals in our experiments and the high inter-individual variation, the differences in LXRα expression between lactation and dry period did not reach statistical significance (Fig. 1). Associations between the mRNA expression of transporters and genes involved in their regulation were also observed for *ABCA7* and *SREBP1*, as well as for *ABCG5* and *SREBP1* (Fig. 2B and 2C). These correlations suggest that *SREBP1* is involved in the regulation of ABCA7 and ABCG5 in the bovine mammary gland. These findings are in agreement with previous reports indicating that ABCA7 is regulated by SREBPs but not by LXR (Iwamoto et al. 2006).

In summary, we gained insight into gene expression patterns in the bovine mammary gland during lactation and subsequent involution by analysing candidate genes that are associated with lipid homeostasis. We found significant differences in the expression patterns of a subset of ABC transporters and potential relationships between selected candidate transporters and their regulatory genes. The better understanding of these transporters and the pathways involved in mammary gland lipid transport may help to elucidate novel molecular mechanisms underlying cholesterol and lipid transfer into milk. To unravel the physiological role and underlying regulatory mechanisms of these lipid transporters in the mammary gland, additional cellular localization studies based on immunohistochemistry or in-situ hybridization are essential. Moreover, the identification of endogenous ligands and substrates in the mammary gland are of fundamental importance.

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